PREVALENCE AND INCIDENCE DENSITY OF MYCOBACTERIUM LEPRAE AND TRYpanosoma CRUZI INFECTIONS WITHIN A POPULATION OF WILD NINE-BANDED ARmadillos

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Abstract. A total of 415 wild 9-banded armadillos from the East Atchafalaya River Levee (Point Coupee, LA) were collected over 4 years to estimate the incidence and prevalence of Mycobacterium leprae and Trypanosoma cruzi and to discern any relationship between the 2 agents. M. leprae infections were maintained at a high steady prevalence rate year to year averaging 19%. T. cruzi antibody prevalence remained relatively low, averaging 3.9%, and varied markedly between years. Prevalence rates were independent, with only 3 armadillos coinfected with both agents. M. leprae incidence density ranged from 0.47 to 3.5 cases per 1,000 animal-days, depending on case definition, confirming active intense transmission of M. leprae among armadillos. No incident T. cruzi cases were found. These infections seem to occur independently and may be used in comparisons to understand better factors that may influence transmission of these agents.

INTRODUCTION

The wild 9-banded armadillo (Dasypus novemcinctus) is well recognized as a host for the human pathogens, Mycobacterium leprae and Trypanosoma cruzi. Despite the importance of these infections to humans, relatively little is known about their epidemiology in armadillos or the role that armadillos may play in human infections. The frequency of human disease caused by these agents, leprosy and Chagas' disease, is rare in North America compared with elsewhere in the Western hemisphere.1–2 High rates of both infections have been reported among armadillos in the United States, however.3–8 Although zoonotic transmission of T. cruzi may be restricted by the absence of appropriate insect vectors in North America, many investigators have speculated that armadillos might be an important source of leprosy infection for humans.3–9 We wondered if there were any similarities in the epidemiology of these infections among armadillos that could aid our understanding of how the agents are transmitted and benefit our appreciation of their zoonotic potential.

Earlier studies confirmed a large reservoir of M. leprae and T. cruzi infections among armadillos and examined the geographic distribution of prevalence rates between small groups of wild armadillos.4–7 Cross-sectional studies based on point prevalence estimates have only limited value.10 Studies relating the disease intensity to factors that influence transmission between animals in different areas may be served better by comparing the prevalence of 2 infections or examining the incidence density of the infections. In this study, we examined the trends in prevalence and incidence density of M. leprae and T. cruzi infections among armadillos in a known endemic Louisiana habitat5 and evaluated those trends over time to discern if there was any relationship between these 2 infections that might influence their local transmission.

MATERIAL AND METHODS

Armadillos and data collection. Armadillos were captured at night with dip nets along the East Atchafalaya River Levee in Point Coupee Parish, Louisiana, between Interstate 90 and 15.7 km north to the Melville Ferry Landing. This capture was conducted under all appropriate animal use requirements of the Office of Laboratory Animal Welfare (assurance no. A3032-01). While in the net, animals were weighed (kg), sexed, and restrained in dorsal recumbence. A 3-ml blood sample was collected aseptically from the subclavian vein with a heparinized Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ). On both ears, a 0.5- to 1-cm full-thickness biopsy specimen was removed aseptically and fixed in 10% buffered formalin. An AVID microchip (AVID Identification System Inc, Norco, CA) was injected subcutaneously in the right side within the cavity between the carapace and the abdominal wall. After implantation, the 9-digit microchip number was identified using the AVID scanner. Before the armadillo was released, the capture position (northing and easting coordinate) and locational data were recorded in the Universal Transverse Mercator coordinate system (metric units) with a 12-channel GPS (Garmin; Olathe, KS) receiver. For incidence studies in 1997, repeat blood and ear samples were collected from armadillos recaptured >21 days from their initial capture. Weight and GPS position also were recorded for the recaptures. GPS data were not available for samples drawn in 1987, 1988, and 1989. These samples were collected in an identical manner, and their M. leprae prevalence was reported previously.3 The sera were stored frozen at –70°C and withdrawn for retesting in this study. The M. leprae and T. cruzi prevalence results were integrated to compare trends in overall yearly prevalence.

Detection of M. leprae infection. Sera were tested in an enzyme-linked immunosorbent assay (ELISA) to detect IgM antibodies to the species-specific phenolic glycolipid-1 (PGL-1) antigen of M. leprae. ELISA procedures were the same as previously reported.5 The antigen (ND-O-BSA) synthetic neoglycoconjugate was supplied by Dr. Patrick Brennan (Colorado State University, Fort Collins, CO) through contract with the National Institute of Allergy and Infectious Disease. Ear tissues were fixed in 10% buffered formalin, and stained sections were examined to detect acid-fast bacilli within dermal nerves using the method previously described.3

Detection of T. cruzi infection. An indirect hemagglutination assay commercially marketed for screening human sera was used to detect antibodies to T. cruzi (Hemagen Chagas
Kit, catalog no. 6419, no. 6421; Hemagen Diagnostics Inc, Waltham, MA). The test with armadillos was performed according to the manufacturer’s instructions except a 13-threshold screening titer was used for initial positive/negative interpretations. Serum samples were screened at a dilution of 1:13, and all positive samples were confirmed with retesting and their titer limits determined to extinction. Individual results were categorized at the 13, 26, 52, and 104 titer settings. Positive detection was determined by observing a standard flocculent pattern within the well as recommended by the manufacturer. Known culture-positive dog sera were provided by Dr. Steve Barr (Cornell University, Ithaca, NY) and used to standardize the assay.

**Incidence density.** In 1997, incidence was measured among armadillos recaptured >21 days after their initial capture date. Incidence density for *M. leprae* and *T. cruzi* was estimated as the number of serologic or histopathologic conversions among recaptured armadillos, divided by the sum of cumulative days (animal-days) between capture and recapture of all recaptured animals. One half of the recapture interval of case animals was allocated to the incidence density denominator. Ninety percent exact confidence intervals (CIs) (Mid-P) of the incidence densities were estimated using PEPI (USD, Inc, Stone Mountain, GA).

**Radius of movement and animal density.** The radius of movement was defined as the average distance between the armadillo’s initial capture and recapture, and this linear distance was calculated using the pythagorean theorem with GPS capture and recapture positions in the northing and easting coordinates. A theoretical area of movement was estimated for each subject by centering a circle, with the radius equal to the estimated radius of movement, on the initial capture position, and the number of armadillos within each given circle was the animal density. Radius of movement and animal density were calculated for the 1997 longitudinal study, and our population estimates were compared with those of previous studies using other techniques.

Geographic information systems software (ArcView; Environmental Systems Research, Inc, Redland, CA) was used in managing the spatial data and performing calculations. A GPS correction was done to reduce random source of error in each animal’s GPS determination by measuring static positions during each hunting period.11 This correction did not reduce any error caused by selective availability.

**Statistical analysis.** The computer programs for the statistical analysis in weight, sex ratio, and prevalence estimates were S-Plus (Mathsoft, Inc, Seattle, WA), StatXact (Cytel Software Corp, Cambridge, MA), and PEPI (USD, Inc, Stone Mountain, GA).

**RESULTS**

**Population features.** A total of 415 wild 9-banded armadillos were collected in the Atchafalaya study area during 4 nonconsecutive years: 1987 (n = 122), 1988 (n = 100), 1989 (n = 28), and 1997 (n = 165). The overall male-to-female ratio of armadillos was 1.15:1 (n = 403, 216 males to 187 females). The sex distribution varied between sampling periods (chi-square, 14; degrees of freedom [df], 3 exact; mid-P = 0.001). The average weight was 4.4 kg (SD, 0.72; n = 378) with a range of 2.2–6.4 kg (Figure 1). Mainly, adult animals were sampled.

The average weight of *M. leprae*-seropositive armadillos was 4.4 kg (SD, 0.71) with a range of 2.3–5.9 kg (Figure 1). The average weight of histopathologically positive armadillos was 4.7 kg (SD, 0.26) with a range of 4.5–5.1 kg. *T. cruzi*-seropositive armadillos weighed on average 4.2 kg (SD, 0.90) with a range of 2.7–5.7 kg. All *M. leprae*-infected and *T. cruzi*-infected animals were adult size.

**Prevalence.** Overall, *M. leprae* seroprevalence combined across the years studied was 19% (79 of 414), and approximate 90% CI (estimated by treating the years as 4 clusters) was 18.4–19.7%. The seroprevalence did not vary significantly between the years studied (chi-square, 0.05; df, 3; P = 0.99). The prevalence rate of *M. leprae* infections determined by histopathologic examination of ear tissue in the 1997 study was 3.0% (5 of 165) and was similar to earlier observations in this habitat.3,12

The overall *T. cruzi* seroprevalence combined across years was 3.9% (16 of 413), and the approximate 90% CI (estimated by the same method as for overall *M. leprae* seroprevalence) was 0.47–7.28%. The year-to-year variation in *T. cruzi* antibody prevalence was statistically significant, but no *T. cruzi*-positive animals were detected in 1 sampling interval (chi-square, 13.0; df, 3; P = 0.001) (Table 1). The *T. cruzi* antibody titers range was 13–104, with 13, the highest, dropping off linearly to 104 (Figure 2). The number of *T. cruzi*-positive animals (n = 16) at each titer level was 16 (13), 11 (26), 6 (52), and 1 (104).

* M. leprae and *T. cruzi* seroprevalence were not correlated (Spearman rank correlation on prevalence value by year, r = 0.4; exact-P = 0.75). Of the 16 *T. cruzi*-positive animals, 3 serologic *M. leprae*-infected armadillos were detected, and none of the 1997 *M. leprae* histopathologic positives were *T. cruzi* seropositive. This finding was consistent with the overall prevalence of both infections within this population. The frequencies of *M. leprae* and *T. cruzi* seropositive animals not associated with one another (common odds ratio estimate across years, 1.0; 95% Mid-P CI, 0.30–4.6).
Incidence density. In the 1997 longitudinal study (n = 165), 5 *M. leprae* serologic incident cases were observed out of 23 enrolled animals. Enrolled armadillos were those recaptured >21 days after their initial capture date. The incidence density of seroconversion was 3.5 cases per 1,000 animal-days (Table 2). The total follow-up times were 574 animal-days (range, 104–125 animal-days per animal). The total follow-up time of the other 18 enrolled was 1,164 animal-days. One *M. leprae* histopathologic incident case was reported (29 enrolled), and the follow-up time for this case was 124 animal-days. The total follow-up time of the other 28 enrolled was 2,082 animal-days. The estimated incidence density for the histopathologic positivity was 0.47 cases per 1,000 animal-days (Table 2). There were no incident cases detected among the *T. cruzi* infections. The total follow-up time of the 23 enrolled armadillos was 1,738 animal days.

Radius of movement and animal density. There were 44 armadillos recaptured, and GPS data on movement were used to examine the density of infections within a given animal’s range (Figure 3). The average radius of movement was 0.16 km (SD, 0.10), the median was 0.14 km, and the range was 0.01–0.50 km. These results were similar to previous estimates of animal movement. The overall mean animal density in the movement area was 3.6 armadillos (SD, 3.3; 95% CI, 2.6–4.6) and ranged from 0–14 armadillos. The animal density of seropositives seemed to be randomly distributed. The average radius of movement of the 5 serologic *M. leprae* incident cases was 0.12 km (SD, 0.06–0.19) with a density of 0–3 armadillos. The 1 histopathologic incident case had a radius of movement of 0.10 km with a density of 4 armadillos. One *T. cruzi*–positive armadillo was recaptured with a radius of movement of 0.19 km and an animal density of 6 armadillos per home range. No clustering effects were noted with regard to infection prevalence, incidence, and animal density.

**Table 1**

<table>
<thead>
<tr>
<th>Year</th>
<th>Number sampled</th>
<th>Percent prevalence</th>
<th>90% Confidence interval</th>
<th>Number sampled</th>
<th>Percent prevalence</th>
<th>90% Confidence interval</th>
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<tr>
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<td>164</td>
<td>2.4</td>
<td>0.8–5.5</td>
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<td>7.3–33.9</td>
<td>28</td>
<td>0</td>
<td>0–10.2</td>
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<tr>
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<td>100</td>
<td>19.0</td>
<td>12.8–26.6</td>
<td>99</td>
<td>1.0</td>
<td>0.1–4.7</td>
</tr>
<tr>
<td>1987</td>
<td>122</td>
<td>18.9</td>
<td>13.2–25.6</td>
<td>122</td>
<td>9.9</td>
<td>5.1–14.5</td>
</tr>
</tbody>
</table>

* *M. leprae* prevalence was not different among the study years (chi-square, 0.05; df, 3; P = 0.99).

† *T. cruzi* prevalence was significantly different among the study years (chi-square, 13.0; df, 3; P = 0.001).

**DISCUSSION**

These data indicate that *M. leprae* and *T. cruzi* are relatively common infections among wild armadillos in Louisiana. Antibodies to both agents were detected among animals throughout the area examined, and prevalence seemed to be independent. Only 3 armadillos were found to have simultaneous infections with *M. leprae* and *T. cruzi*, suggesting that these agents probably do not share any common mechanisms of transmission or special epidemiologic relationships. *M. leprae* prevalence remained steady year to year, and our incidence density estimate shows that *M. leprae* is intensely transmitted among armadillos within this region. *T. cruzi* antibodies were less prevalent and more variable in the population from year to year.

*M. leprae* causes a slow chronic infection that is detected mainly in adult armadillos. The disease can be diagnosed with serologic or histopathologic methods. *M. leprae* is the only mycobacterial pathogen that invades nerves, and detection of acid-fast bacilli in dermal nerves is diagnostic for leprosy. This manifestation is found only in the latest stages of disease in the armadillo, however, and serologic methods can...
show positive results much earlier. Although the mycobacteria share many cross-reactive antigens, *M. leprae* can be differentiated on the basis of its species-specific PGL-1 antigen. Among armadillos experimentally infected intravenously with $1 \times 10^9$ leprosy bacilli, 6–9 months usually elapse before IgM antibodies to PGL-1 become detectable. Once present, however, these antibodies tend to achieve high titers and remain detectable over the course of infection.

Because armadillos reach sexual maturity around 18 months of age, most endemic leprosy infections are detected among adult animals, and circumstances that can influence the age structure of a given population can influence markedly the detectability of leprosy in that group. Considerably less is known about the antibody response of armadillos to *T. cruzi*. Different strains of *T. cruzi* seem to evoke widely different serologic and pathologic responses in different hosts. We found *T. cruzi* antibodies across nearly all weight classes. Their titer or frequency did not increase with weight, and their prevalence varied from year to year. The *T. cruzi* strain infecting armadillos in this habitat seems to attack all age groups acutely and evokes a more transient antibody response compared with *M. leprae*.

Armadillos in the Louisiana Atchafalaya Basin first were surveyed for sylvatic leprosy in the 1980s, and the high steady seroprevalence of *M. leprae* infection described here is in keeping with those earlier estimates. Earlier estimates on the prevalence of *T. cruzi* infection among armadillos in Louisiana are quite disparate, however. In an earlier study on armadillos taken from this same locale, Barr et al. found 1.1% of animals to be culture positive for *T. cruzi*, a rate similar to the 3.5% antibody prevalence we describe here. In an adjacent area approximately 50 miles away, Yaeger estimated that 28% of the animals were burdened with *T. cruzi* based on direct isolation of trypomastigotes and serologic agglutination. It is unclear if these disparities result from differences in the local environment or in the diagnostic methods used. Yaeger showed that all of the culture-positive samples also were positive by serologic agglutination. He used a threshold dilution of only 1:4, however, which may have yielded false-positive reactions in many of the culture-negative animals. Direct isolation of *T. cruzi* from whole blood may be the preferred screening method. It is time-consuming and has variable efficiency in different hands, however. With whole blood not being available in our retrospective study, the indirect hemagglutination assay was chosen for a rapid economical screening method applicable to this exotic animal species. Other studies have shown the indirect hemagglutination assay to have good correlation with the other commercially available radioimmunoprecipitation, enzyme-linked immunosorbent, and immunofluorescence assays for *T. cruzi* antibody.

The incidence density of *M. leprae* infections in wild populations has never been shown. Such studies require a suitably high prevalence and a large number of animals available for sample and recapture, a circumstance that is often quite problematic when dealing with medium and large-sized, free-ranging animals. Our serologic and histopathologic incidence density estimates, 3.5 and 0.47 per 1,000 animal-days, show a great deal of *M. leprae* transmission within the studied population. This is equivalent to an average of 1.3 seroincident cases per animal year of risk, or an annual probability of infection equal to 0.72. With the long latency of *M. leprae*, the observed incident cases probably were harboring *M. leprae* at their initial capture, but a detectable immune response had not yet fully developed. By the time the incident cases were recaptured, their immune responses progressed to the point of detectability. In contrast to *M. leprae*, however, no incident cases of *T. cruzi* were detected, an observation consistent with the low *T. cruzi* infection prevalence measured in the population.

Geographical information systems with satellite positioning afford us the opportunity to assess individual animal interactions and explore the importance of contact in transmitting infections. The individual animal movements and densities seen here varied throughout the habitat but were in keeping with previous population-based estimates for this location. Although no nidality of *M. leprae* or *T. cruzi* infections was visually detectable on our map of the capture area, this study showed the potential to conduct spatial and temporal incidence studies. Further work should explore sources of random or systematic error in our method for measuring animal movement and locations.

These results support the conclusion that wild 9-banded armadillos harbor *M. leprae* and *T. cruzi*, but no relationship between these agents seems to exist. *M. leprae* is transmitted intensely among wild 9-banded armadillos in this area. Although evidence of *T. cruzi* infections was found, prevalence was lower than that estimated in another wild armadillo population in Louisiana. With the ability to estimate *M. leprae* incidence density, we can conduct longitudinal studies of risk factors for natural infection and perhaps open the door to an improved understanding of leprosy transmission in humans.

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